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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Richard Wurtman, Ph.D. Examiner: L. E. Crane, Ph.D.

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For: METHODS FOR TREATING CYTIDINE LEVELS AND METHODS FOR TREATING CYTIDINE-DEPENDENT HUMAN DISEASES

DECLARATION UNDER 37 C.F.R. §1.132

Commissioner for Patents
Washington, DC 20231
Sir:

I, Prof. Richard J. Wurtman, Ph.D., hereby make the following Declaration:

1. I am a co-inventor of the above-identified application and currently hold the positions Cecil H. Green Distinguished Professor MIT, and Director, General Clinical Research Center, Harvard-MIT Division of Health Science and Technology. I am the same Richard J. Wurtman who presented an earlier Declaration, signed September 22, 2003.

2. I understand that at the interview held on January 20, 2004, Examiner Crane questioned whether one of ordinary skill in the art would have appreciated the significance of FIG. 4 in the above-identified application. Specifically, the Examiner was uncertain whether such a person would have (1) expected the results shown in FIG. 4, and (2) understood the significance of those results for enhancing neurological functions, at the time the invention was made.

3. FIG. 4 shows the percentage enhancement of both uridine and cytidine levels in the gerbil brain 60 minutes after oral administration of 250 mg/kg of uridine by gavage (i.e., a known amount, in solution, was inserted into their stomachs by stomach tube). The data in FIG. 4 relate to administration of uridine. However

these results are also representative of uridine mono-phosphate (UMP) administration, because UMP is quantitatively hydrolyzed to uridine by enzymes in the intestinal mucosa before entering the blood.

4. Gerbils were selected for this experiment over rats and other rodents for several reasons. Among available mammals, gerbils are widely accepted as useful models for evaluating treatments for neurological disorders, such as stroke, epilepsy, and age-related memory loss. (Vincent, GE et al., *Lab Animal Sci* 1979, 29:645-51 and Cheal M, *Exp Aging Res* 1986, 12:3-21). Also, the pyrimidine metabolism of gerbils is more representative of humans than rats. For example, human plasma cytidine levels (< 0.1 μM) are lower than plasma uridine levels (4.5 μM). The same relative order is found in gerbils, i.e. 10 μM plasma uridine and 4 μM plasma cytidine. The opposite is order is found in rats, which have nearly twice as much plasma cytidine as plasma uridine. This difference results from higher activity of cytidine deaminase, the enzyme that converts cytidine to uridine, in humans than rats (Camiener GW and Smith CG, *Biochem Pharm* 1965, 14:1405-16).

5. The results in FIG. 4 surprisingly indicated that uridine, once transported into the brain, is converted into cytidine in significant amounts; brain cytidine levels rose 39%. In addition, it showed that brain uridine levels doubled over control values, which had clear implications for enhancing memory.

6. At the time these experiments were being done, it was not apparent to those of skill in the art that brain uridine is converted into cytidine, yet our results demonstrated an increase in brain cytidine following the sole administration of uridine. Where brain cytidine levels had been experimentally or therapeutically elevated in the past, uridine had been used in conjunction with other compounds (e.g., cytidine) that could have accounted for the observed rise in cytidine (Monticone GF, *Minerva Med*, 1966 Dec. 19; 57(101): p4348); or, uridine itself was not used at all (e.g., CDP-choline; Spiers PA, *Arch Neurol*, 1996; (53) p441). A person of ordinary skill in the art would not have predicted the results shown in FIG. 4 in view of these references.

7. We were the first to demonstrate not only that brain cytidine levels could be elevated with uridine, but that endogenous levels of cytidine could be raised

significantly, i.e., 39%. Previous methods for increasing brain cytidine levels had yielded a rise of less than 10%. (Dawson, *J Neurochem*, 1968; (15) p31).

8. The results in FIG. 4 also indicated to a person of skill in the art that uridine or a uridine phosphate would offer an advantage over the use of a cytidine source alone (e.g., CDP-choline) for enhancing neurological functions, because *both* brain cytidine and brain uridine are eventually converted into cytidine triphosphate ("CTP"), which controls the rate of neuron membrane synthesis (discussed below). Uridine is phosphorylated to uridine triphosphate ("UTP"), then UTP is converted to CTP, while cytidine is directly phosphorylated to CTP. These two pathways of CTP synthesis do not compete with each other. Moreover, uridine crosses the blood-brain barrier more effectively than cytidine.

9. Raising the brain CTP level was known to increase neuron membrane synthesis because the CTP level controls the rate at which brain neurons produce phosphatides, such as phosphatidylcholine ("PC") and phosphatidylethanolamine ("PE"), which are the major constituents of brain membranes. Normally, the amount of CTP in neurons is not sufficient to sustain maximal rates of phosphatide production. CTP levels in the brain are only 100 micromolar, whereas the enzyme converting CTP to CDP-choline for phosphatide synthesis requires more than 10 times that amount to reach half its maximal rate of synthesis. (Brian Ross, Anna Moszczynska, Jan Blusztajn, Allan Sherwin, Andres Lozano & Stephen Kish Phospholipid Biosynthesic Enzymes in Human Brain; *Lipids*, 32:351-358, 1997).

10. Hence a treatment that increases CTP levels would tend to increase the formation of brain membranes like the synaptic membranes responsible for communications between neurons. Therefore, with regard to enablement of the present method for enhancing memory, a person of ordinary skill in the art would have understood, when the invention was made, that enhancing brain cytidine levels in general should improve memory, and that a 39% increase in brain cytidine would likely be sufficient for that purpose.

11. We had previously shown that when aging human subjects were given a cytidine source (i.e., CDP-choline), some of their memory deficits were ameliorated (Spiers PA, *Arch Neurol*, 1996; (53) p441). In view of the results shown in FIG. 4, giving such patients uridine or a uridine phosphate, such as UMP, should be even

more effective for the reasons discussed in paragraphs (5) to (8). UMP is preferred for oral administration because it is generally recognized as safe (i.e., it is on the GRAS list maintained by FDA).

12. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: Feb. 4, 2004

By: RJ Wurtman

Prof. Richard J. Wurtman, ~~Ph.D.~~ M.D.